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**PATENT**
**Attorney Docket No.: 050179-0080**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**



In re Application of )
)
Himanshu N. BRAHMBHATT, et al. )
)
Serial No.: 09/530,772 )
)
Filed: May 04, 2000 )
)
For: SUICIDE EXPRESSION VECTOR FOR USE )
IN VACCINE STRAINS )

Group Art Unit: 1655

Examiner: Chakrabarti, A.

**AMENDMENT AND REPLY PURSUANT TO 37.C.F.R. §1.111**

Honorable Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Office Action [non-final rejection] mailed February 21, 2001, kindly amend the above-captioned patent application as follows:

**IN THE CLAIMS**

Please amend the claims as follows:

2. The vector according to claim 1, wherein the first nucleotide sequence encodes an antigen, enzyme or toxin.

3. The vector according to claim 2, wherein the first nucleotide sequence encodes a contraceptive antigen.

4. The vector according to claim 2, wherein the first nucleotide sequence encodes an esterase capable of hydrolyzing organophosphates.

5. The vector according to claim 2, wherein the first nucleotide sequence encodes an insecticidal toxin.

Sub D3

6. The vector according to any one of the preceding claims, wherein the second nucleotide sequence encodes a restriction enzyme or functional portion thereof that recognizes a cleavage site(s) of ten or more nucleotides.

7. The vector according to claim 6, wherein the second nucleotide sequence encodes a restriction enzyme selected from the group consisting of I-*Ppo*I, I-*Ceu*I, P1-*Psp*I, P1-*T1i*I and P1-*Sce*I.

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8. The vector according to any one of the preceding claims, wherein the one or more cleavage sites(s) is/are located at a site(s) on the vector which avoids steric hindrance of binding by said restriction enzyme or functional portion thereof.

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9. The vector according to any one of the preceding claims, further comprising a third nucleotide sequence encoding a ribozyme targeted against mRNA produced from the said second nucleotide sequence encoding the restriction enzyme or functional portion thereof.

10. The vector according to any one of the preceding claims, wherein the second promoter is selected from the group consisting of the p*lac*Z promoter, the p*lacUV5* promoter and the T7 RNA polymerase promoter.

11. The vector according to claim 10, wherein the second promoter is the T7 RNA polymerase promoter.

12. The vector according to claim 11, further comprising an additional nucleotide sequence encoding T7 RNA polymerase operably linked to a third promoter sequence, said third promoter sequence being inducible.

14. The host cell according to claim 13, wherein said host cell is a bacterium or yeast.

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## REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.112, and in light of the remarks which follow are respectfully requested.

By the present amendments, the claims have been amended in an effort to expedite prosecution. These amendments should not raise any issues of new matter.

Turning now to the Office Action, Claims 1 through 19 are rejected under the second paragraph of 35 U.S.C. §112 as assertedly being indefinite. These rejections are respectfully traversed to the extent they may be applicable to the claims as amended and for at least the following reasons.

Specifically Claims 1-16 stand rejected over the recitation of the phrase, "heterologous peptide." Applicants submit that a person of ordinary skill in the art would understand that the term "heterologous peptide" refers to peptides which are not native to the "selected host cell." Further, Applicants bring the Examiner's attention to page 5, lines 16 through 18 of the specification, which set forth examples of the heterologous peptide, polypeptide or protein including *Zona pellucida*, sperm, hormone antigen, an esterase or an insecticidal toxin. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 2 through 12, and 14 are rejected as being indefinite for insufficient antecedent basis. Accordingly, Applicants have amended the claims and withdrawal of this rejection is therefore respectfully requested.

Claims 15 through 19 stand rejected as being indefinite because the instantly claimed methods allegedly lack a final process step that clearly relates back to the preamble. Applicants submit that the claimed methods are not indefinite and that one of ordinary skill in the art would

understand the scope of the claims. In both Claims 15 and 17, the desired products of the claimed methods, respectively, are the "heterologous peptide, polypeptide or protein," and a "microorganism vector" containing the "heterologous peptide, polypeptide or protein." In both Claims 15 and 17 the resulting product is a "suicide expression vector" which has sufficient antecedent basis in the claims, and which contains the pertinent "heterologous peptide, polypeptide or protein." Accordingly, the rejection of Claims 15 through 19 under 35 U.S.C. §112 should be withdrawn and such favorable action is respectfully requested.

Claims 1, 2, 6, 8 and 13 through 19 stand rejected under 35 U.S.C. §102(b) as assertedly being anticipated by Herrero *et al* (Journal of Bacteriology, (1990), Vol. 172, No. 11, pages 6557-6567). This rejection is traversed for at least the following reasons.

Applicants respectfully traverse the Examiner's conclusion that Herrero *et al* anticipates the claimed invention. It is respectfully submitted that Herrero *et al* does not disclose each and every element of Applicant's invention. Herrero *et al* (1990) discloses a transposase to randomly integrate genes into the chromosome of target bacteria. The transposase is not and could not be used in the same way as the restriction enzyme is used in the present application. In the present patent application, the restriction enzyme is encoded by a second nucleotide sequence on the vector and targets a specific cleavage site(s) that is on the vector and not in the chromosome of the host cell.

In addition, Herrero *et al* also refers to a "suicide plasmid;" however the suicide capabilities of the plasmid are not related to the presence of the transposase on the plasmid and are very different to what is described in the present application. That is, in Herrero *et al*, the plasmid is not stable without the presence of the *pir* gene in the chromosome of the host cell. Thus in a cell that doesn't contain that gene, the plasmid is quickly lost. In contrast, the suicide

expression vector of the present invention may be stable in the host cell until desired. That is, the present invention allows expression of a suitable amount of a heterologous peptide, polypeptide or protein encoded by a first nucleotide sequence to accumulate in the host cell. Then, an induction agent can be used to cause the expression of the restriction enzyme to cleave the suicide expression vector (which is then subsequently degraded in the host cell). Therefore, the present invention allows for the complete removal of heterologous DNA (i.e. the suicide expression vector) from a host cell, which, as indicated in the "background" section of the present specification, is clearly desirable. In Herrero *et al* however, recombinant (i.e. heterologous) DNA will remain in the chromosome of a host cell.

Thereby, Herrero *et al* lacks at least three elements of Applicants' suicide expression vector, host cell and method claims, including plasmid stability, an inducible restriction enzyme, and removal from the host of the heterologous DNA. Thus, the rejection under §102(b) based on Herrero et al should be withdrawn and such favorable action is requested.

Claims 1, 2, 6, 7, 8 and 13 through 19 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Herrero *et al* in view of Marshall *et al* (U.S. Patent No. 5,420,032). This rejection is respectfully traversed for at least the following reasons.

Initially, we submit that there is no motivation for a person skilled in the art to combine the disclosure of Herrero *et al* with that of Marshall *et al.* As indicated above, Herrero *et al* is directed to transposon vectors for stable chromosomal insertion of foreign genes in bacteria, while Marshall *et al* is directed to a novel restriction endonuclease. These documents appear to contain different subject matter and it is unlikely that a person skilled in the art would obtain and read the documents together. To do so, clearly involves the impermissible benefit of hindsight.

Notwithstanding, combining Herrero *et al* with Marshall *et al* would not place a person skilled in the art in possession of the present invention. Simply, Herrero *et al* does not describe the use of a restriction enzyme to cleave a vector at a specific cleavage site and Marshall *et al* only describes a restriction endonuclease I-*Ceu*I that cleaves at a specific cleavage site defined by a 15, 17 or 19 base pair sequence (see column 5 line 7 to column 6 line 2). Marshall *et al* does not mention or suggest a vector that codes for both the restriction enzyme and its target cleavage site, nor that the expression of the restriction enzyme may be induced. Further, it is an important feature of the present invention that the restriction enzyme is selected so that it is non-deleterious to the host cell. Neither of the cited references, mention or suggest such a selection and, indeed, in Marshall *et al*, expression of the restriction enzyme I-C*eu*I has been demonstrated to be highly toxic to *E. coli* (see column 8 lines 25-28). Accordingly, this rejection under §103(a) should be withdrawn and is hereto respectfully requested.

Turning to item 7 at page 8 of the Official Action, Claims 1, 2, 6, 7, 8 and 13 through 19 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Herrero *et al* in view of Hardy *et al* (U.S. Patent No. 5,851,817).

It is submitted that Herrero *et al* and Hardy *et al* when combined do not describe the present invention. Hardy *et al* provides DNA segments that encode mammalian sperm proteins that bind to eggs in a species specific manner. Hardy *et al* disclose that the DNA segments of interest may be positioned under the control of a promoter, and particularly, a recombinant promoter, in order to create a recombinant vector such as a recombinant expression vector. The vector described by Hardy *et al* (expressing a contraceptive antigen) is not a suicide expression vector that utilizes inducible expression of a restriction enzyme to cleave the vector and thus

cause its removal. Therefore, Herrero *et al* and Hardy *et al* combined, do not describe or suggest the present invention. Thus, withdrawal of this rejection is hereby respectfully requested.

With regard to items 8, 9, 10 and 11 of the Official Action, it is similarly submitted that the combination of Herrero *et al* with, respectively, Calvet *et al* (U.S. Patent No. 5,552,313), Kemp *et al* (U.S. Patent No. 6,111,070), Barber *et al* (U.S. Patent No. 6,043,077) or Cameron *et al* (U.S. Patent No. 6,143,518) does not disclose or suggest the present invention. These rejections are respectfully traversed for at least the following reasons.

As presented above, Herrero *et al* describes transposon vectors for stable chromosomal insertion of foreign genes in bacteria. In contrast, the present invention relates to a suicide expression vector that can be used to express a heterologous peptide (not native to the host cell) from a first nucleotide sequence and enables removal of the vector when required by expression of a restriction enzyme, from a second nucleotide sequence, wherein the restriction enzyme specifically cleaves a target cleavage site on the vector.

Notwithstanding, Calvet *et al*, Kemp *et al*, Barber *et al*, and Cameron *et al* fail to cure the deficiencies of Herrero *et al*.

Calvet *et al* describes a vector that expresses a phosphotriesterase-related protein. This document in no way describes the expression of this protein from a suicide expression vector. Calvet *et al* does not assign any enzymatic function to this protein, let alone an esterase function that hydrolyses organophosphates (i.e. cleaves DNA).

Kemp *et al* describes a vector that can be used to express foreign proteins in plant cells. Kemp *et al* discusses the expression of insecticidal proteins from this vector but does not describe a suicide expression vector.

Barber *et al* describes ribozymes, and ribozyme delivery, for the treatment of Hepatitis C Virus. Claim 9 of the present application recites the use of a ribozyme targeted to mRNA transcribed from the restriction enzyme nucleotide sequence. As disclosed at page 4 lines 6 to 15 of Applicants' description, the suicide expression vector preferably further comprises a nucleotide sequence encoding the ribozyme as a "safeguard" against premature expression of the restriction enzyme or functional portion thereof. By contrast, Barber *et al* use the ribozyme to target an RNA molecule that is not produced from the vector but from a virus. Barber *et al* does not describe the use of a ribozyme to cleave low "leakage" amounts of mRNA encoding a restriction enzyme or functional portion thereof. Furthermore, Barber *et al* in no way describes the use of a suicide expression vector for expression of this ribozyme.

Finally, Cameron *et al* describes a vector for expression of recombinant proteins which is more stable in *E. coli* than previous vectors due to the presence of a *par* region. This plasmid is clearly not a suicide plasmid expression vector.

In summary, it is submitted that none of the documents cited by the Examiner taken together or alone describe or suggest the present invention. The vector described in the present application enables expression of a heterologous peptide, polypeptide or protein in a host cell and can then be removed from the host cell as desired. This is achieved by inducing, when required, expression of a restriction enzyme that targets a specific cleavage site in the vector. Expression of the restriction enzyme results in cleavage of the vector and subsequent degradation and removal of the vector from the host cell. In a preferred embodiment, a third nucleotide sequence is provided in the suicide expression vector which encodes a ribozyme targeted against mRNA transcribed from the restriction enzyme nucleotide sequence and is used to reduce background (or "leaky") expression of the restriction enzyme prior to induction.

Therefore, based on the foregoing, withdrawal of the §103 rejections based on Herrero *et al* in view of respectively, Calvet *et al*, Kemp *et al*, Barber *et al*, and Cameron *et al* are respectfully believed to be in order, and such favorable action is respectfully requested.

In conclusion and based on the preceding, this application is believed to be in condition for allowance. A notice to that effect is respectfully solicited. However, if any issues remain outstanding, the Examiner is respectfully requested to contact the undersigned so that prosecution may be expedited.

Date: 6/21/01

Respectfully submitted,

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